Effect of microbial activity, soil water content and added copper on the temporal distribution patterns of HCB and DDT among different soil organic matter fractions

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Biological activity, soil moisture and Cu have different effects on HCB and DDT transfer from FA, HA and BHA to lipid and IR fractions.

Abstract

Temporal changes in the distribution of exogenous HCB and DDT among different soil organic matter fractions were studied under sterile and non-sterile conditions, different soil water contents, and different concentrations of added Cu^{2+} . The residence time was 311 days. Soil organic matter was fractionated into fulvic acid (FA), humic acid (HA), bound-humic acid (BHA), lipid, and insoluble residue (IR) fractions by a methyl isobutyl ketone (MIBK) method. Results revealed that there is a mass transfer tendency of DDT and HCB from FA, HA and BHA to IR and lipid fractions with increasing residence time. Microbial activity accelerated the mass transfer, while the addition of Cu^{2+} slowed it down. The HCB and DDT transfer rate decreased as the soil moisture increased from 1.9% to 60%, but increased when soil moisture increased further to 90%. A two-compartment first order kinetic model was used to describe the mass transfer from FA, HA and BHA.

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1. Introduction

Hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) are of great concern as typical hydrophobic organic pollutants (HOCs) due to their long half-life, bioaccumulative nature and chronic adverse effects on humans and animals. They have been detected in air, soil, fish, birds, and even in human milk (Qiu et al., 2004). The sequestration of DDT and HCB is of considerable interest because of its effect on bioavailability and the risk-assessment paradigms used to guide management and cleanup of contaminated sites and soil. The processes of HOCs sequestration in soil are thought to be driven by partitioning into the soil organic matter (SOM)

(Gustafsson et al., 1997; Cornelissen et al., 2005) and diffusing into soil micropores (Farrell et al., 1999). In soil with greater than 0.1% organic carbon content, partitioning into SOM has been found to be the dominant process (Chiou et al., 2000; Kang and Xing, 2005).

Among the SOM fractions, humin is considered to be the most important one (Kohl and Rice, 1998; Macleod and Semple, 2003). Several investigations deemed that as residence time increases, HOCs tend to transfer from FA and HA fractions to humin (Weber and Huang, 1996; Bogan and Trbovic, 2003). Moreover, HOCs associated with humin exhibit more resistance to desorption and less bioavailability (Chiou et al., 2000; Nam and Kim, 2002; Kang and Xing, 2005). In our previous study (Zhang et al., 2007), a MIBK isolation technique (Kohl and Rice, 1998) was applied to fractionate SOM into fulvic acid (FA), humic acid (HA) and humin fractions, and then

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humins were further isolated into bound humic acid (BHA), lipid and insoluble residue (IR) fractions. The CPMAS ^{13}C NMR spectra of HA, BHA, lipid, and IR convincingly revealed different carbon-type distributions among these humic fractions. Moreover, as residence time increased, the contents of DDT and HCB in the FA, HA and BHA fractions decreased, while those in the IR and lipid fractions increased.

Besides soil organic type and content (Bogan and Trbovic, 2003; Kang and Xing, 2005), many environmentally relevant factors affect mass transfer of HOCs in soil, such as hydrophobic porosity (Nam and Alexander, 1998), microbial activity (Carmichael et al., 1997; Macleod and Semple, 2003), soil moisture (Gupta and Gajbhiye, 2002), soil aggregation (Nam et al., 2003) and co-existing metal ions (Yuan and Xing, 2001; Feng et al., 2006). The effect of microbial activity on the mass transfer of HOCs was ascribed to partial transformation and/or carbon turnover activities of the soil microbial communities (Guthrie and Pfaender, 1998; Macleod and Semple, 2003). Water is the medium that organic contaminants diffuse to binding sites within soil aggregates, and may have a particularly complicated effect on the mass transfer of organic compounds in soils (Gaillardon and Dur, 1995; Pignatello, 1998). Soil water not only competes for adsorption on soil minerals (Gaillardon and Dur, 1995), but also changes the properties of SOM (Pignatello, 1998), hence influences the affinity of SOM for HOCs, desorption hysteresis and sequestration of HOCs (Xing and Pignatello, 1997). Heavy metals and organic contaminants often coexist in soils. The negatively charged surfaces of humic materials tend to bind metal ions, which reduce the negative charge and contribute to an improvement in the hydrophobic property of humic substances (Saison et al., 2004). Moreover, metal cations often encourage humic substances to adopt a coiled configuration and possess glassy properties, leading to greater sorption capacity and non-linearity, and higher desorption hysteresis (Yuan and Xing, 2001; Wrobel et al., 2003; Feng et al., 2006).

Although extensive evidence indicated that microbial activity, water content and coexisting metals affected the sequestration of HOCs, there is little information on the effects of those factors on the temporal distribution pattern of HOCs among various SOM fractions. Therefore, the aim of this work was to study the temporal distribution of HCB and DDT among various SOM fractions as affected by those environmentally relevant factors to better understand the sequestration process of HCB and DDT in soils. The two-compartment first order kinetic model was used to describe the transfer of HCB and DDT from each SOM fraction. The implications of such temporal distribution change for the soil environment are discussed.

2. Materials and methods

2.1. Soil samples

Soil used in this study was collected from Heilongjiang province of China (Isohumisols, 5.48% organic carbon, pH 7.35). The soil was air-dried, ground, and passed through a 2-mm sieve. The air-dried soil contained 1.9% of water content, and background Cu content of soil was 10.2 mg kg^{-1} .

Cation-exchange capacity (CEC) determined by the BaCl_2 method (Hendershot and Duquette, 1986) was $25.6 \text{ cmol kg}^{-1}$. All subsoil samples were sealed in glass bottles and stored at room temperature for subsequent analysis and experiments.

2.2. HCB and DDT spiking and aging

2.2.1. Experiment 1: study on the effect of microbial activity

One hundred grams of air-dried and sterilized (by ^{60}Co irradiation, 2.5 Mrad) soil samples in triplicate were spiked with $200 \mu\text{g}$ of HCB and DDT in 10 ml hexane, blended and placed in a hood to evaporate hexane for 5 h. Non-sterilized soil samples treated with the same spiking were used for comparison.

2.2.2. Experiment 2: study on the effect of soil water

The second 100 g of air-dried and sterilized soil samples in triplicate spiked with $200 \mu\text{g}$ of HCB and DDT in 10 ml hexane as described above were saturated with sterilized distilled water to bring the moisture level to 30%, 60% and 90% of its water holding capacity, respectively. During the course of this study, sterilized distilled water was added to compensate for the loss of water.

2.2.3. Experiment 3: study on the effect of added Cu^{2+}

Various amounts of Cu solution ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, guaranteed reagent grade) were added to the third 100 g of air-dried and sterilized soil samples in triplicate to 200, 500 and $1000 \mu\text{g g}^{-1}$ Cu, respectively. The concentrations of Cu added represent the Cu-contaminated soil from low to high levels (Wang et al., 2007; Niklińska et al., 2006). After mixed thoroughly, the soils were spiked with $200 \mu\text{g}$ of HCB and DDT in 10 ml hexane and evaporated as described above.

All the soil samples were sealed in solvent-rinsed microcosms (1-kg Kilner jar) and sterilized, and then incubated at 20°C and stored in the dark for different time intervals. During the incubation time, the soils were sterilized every 2 months to maintain the sterility.

2.3. Fractionation of SOM substances

SOM were operationally fractionated into FA, HA, BHA, lipid, and IR fractions according to the MIBK technique described by Kohl and Rice (1998). The whole procedure is summarized in Fig. 1.

Briefly, 10 g incubated soil sample was first mixed with 200 ml of 0.5 M NaOH and shaken under nitrogen for 24 h. The sample was centrifuged for 30 min and the supernatant containing FA and HA was separated. The residue precipitate was then briefly stirred with an additional 50 ml of 0.5 M NaOH and centrifuged for 30 min. The supernatant containing FA and HA was

Fig. 1. Sequence of steps in the MIBK method.

combined with FA and HA obtained from the previous procedure. This stir and centrifugation operation was repeated five times. FA and HA were further separated by acidifying to a pH < 2 with concentrated HCl and centrifuging. The aqueous phase was referred to as FA and the precipitate was HA.

The humin fraction with soil residue was transferred to a separatory funnel along with 200 ml of de-ionized water and 200 ml of MIBK. The mixture was acidified to about pH 2 by concentrated HCl, shaken vigorously for about 1 min, and allowed to equilibrate for about 1 h. The aqueous phase containing FA and precipitate was separated from MIBK. Then FA and precipitate were further separated from each other by centrifuging, and FA was combined with the FA fraction obtained above. The residue precipitate was transferred with 200 ml of 0.5 M NaOH to the same separatory funnel, shaken vigorously and allowed to stand again for 1 h. BHA entered the alkaline aqueous phase, and was then separated from the precipitate. The precipitate was returned to the separatory funnel again with 200 ml of de-ionized water, shaken vigorously for about 1 min, and allowed to equilibrate overnight. The aqueous phase and precipitate were discharged, and further separated from each other by centrifugation. BHA in the supernatant was combined with the BHA fraction obtained above. The precipitate contained the IR fraction. The MIBK phase contained the lipid fraction.

2.4. Analysis of organic carbon in each SOM fraction

The organic carbon contents of the FA and BHA fractions were analyzed with a Phoenix 8000 total organic carbon analyzer (Tekmar-Dohrmann Co., Cincinnati, OH, USA) after residual MIBK had been removed by evaporation. While those of the HA, lipid and IR fractions were determined by the Walkley–Black titration (Nelson and Sommers, 1982) after evaporation of all organic solvent or water.

2.5. Determination of HCB and DDT in SOM fractions

HCB and DDT in the soil, HA, BHA and IR fractions were extracted by the Soxhlet extraction method. Frozen dried HA, BHA or IR fraction, or 2.00 g of soil sample was ground with 10.00 g of anhydrous Na₂SO₄ in a mortar and pestle prior to Soxhlet extraction. The ground mixture was placed in a thimble filter and extracted with 100 ml of 1:1 (v/v) hexane/acetone for 24 h at 5–6 min cycle⁻¹. HCB and DDT in FA were extracted three times with 200 ml of hexane in a separatory funnel for 24 h (Nam and Kim, 2002).

The extracts and MIBK were concentrated to about 1–2 ml by a rotary evaporator, and further purified with a chromatographic column (15 mm length × 10 mm i.d.) loaded with 10 g activated Florisil (60–100 mesh, Wenzhou Chemical Reagent Factory, China). The elution was subsequently carried out using 100 ml hexane containing 10% acetone (v/v). After concentrating to near dryness in a rotary evaporator, the elution was diluted with *n*-hexane, and brought to exactly 1.0 ml under a gentle stream of pure nitrogen for gas chromatographic analysis.

The concentrations of HCB and DDT were analyzed with a GC (Agilent 6890N, Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a Nickel 63 electron capture detector (μECD) and a HP-5 fused silica capillary column (30 m length × 0.32 mm i.d. × 0.25 μm film thickness, J&W Scientific Co., Folsom, CA, USA). The oven temperature program started at 60 °C (holding time 1 min), increased to 140 °C at 15 °C/min, and finally programmed to 280 °C at 8 °C/min (holding time 5 min). The temperature of the injector and detector was 220 and 300 °C, respectively. High purity nitrogen was used as both carrier (2.0 ml min⁻¹) and makeup (60.0 ml min⁻¹) gas. Samples (1 μl) were injected under splitless injection mode. The concentrations of HCB and DDT were determined by comparing the peak area of the samples with the calibration curves of the standards.

2.6. Quality control and quality assurance

Strict quality control was employed for the extraction and analysis. Decachlorobiphenyl (DCBP) was added to the fractionated samples before HCB and DDT extraction and purification as a surrogate. Recoveries of the surrogate standards from the FA, HA, BHA, lipid and IR fractions were 89–96%, 92–98%, 84–91%, 97–99%, and 91–95%, respectively.

Standards of HCB (C₆Cl₆, purity 99.9%) and DDT (2,2-bis-(4-chlorophenyl)-1,1,1-trichloroethane, purity 98%) were purchased from Aldrich Inc. (USA), and used as received without further purification. All reagents used were of pesticide grade. The concentrations of HCB and DDT were quantitatively determined by external standard method using peak area mode with 2,4,5,6-tetrachloro-*m*-xylene as internal standard added before GC-ECD determination. The detection limits (DL) of HCB and DDTs were determined at signal-to-noise ratio (*S/N*) of 3. The HCB and DDT recoveries were determined based on the peak area ratio of direct injection of extract to the working standards prepared in hexane.

The concentrations of HCB and DDT in soils and each soil fraction were determined immediately after spiking and solvent evaporated. The average recoveries of HCB and DDT ranged from 92% to 101% and 90% to 98%, respectively, indicating the accuracy and homogeneity of the spiking. The relative standard deviation (RSD) values of the contents of HCB and DDT in six replicated fractionations were in the range of 2.4–16.3%. The instrument detection limits of HCB and DDT were 0.04 and 0.1 μg l⁻¹, respectively. The sum of the HCB and DDT in the five fractions was comparable with the total HCB and DDT contents obtained by Soxhlet extraction of the soils, showing good reproducibility of the fractionation and determination performance. The data confirmed the practicability of the fractionation and analytical protocols of HCB and DDT in various SOM fractions and in soils.

2.7. Transfer kinetics model

A two-compartment first-order kinetic model was used for the description of mass transfer of HCB and DDT from the FA, HA and BHA fractions (Watanabe et al., 2005).

$$\frac{q(t)}{q_0} = \Phi_r \exp(-k_r t) + (1 - \Phi_r) \exp(-k_s t) \quad (1)$$

where Φ_r is the percentage of HCB or DDT of the rapid transfer fraction controlled by expanded SOM region, while $(1 - \Phi_r)$ is the percentage of the slow transfer fraction controlled by condensed region, k_r and k_s are the corresponding apparent first order rate constants of rapid and slow transfer, respectively.

3. Results and discussion

3.1. Spiking of HCB and DDT to soils

Over the given residence time intervals of 1, 7, 14, 30, 90, 120, 184 and 311 days, the recoveries in the sterilized soil were 92–99% and 88–96% for HCB and DDT, respectively, suggesting that the loss and degradation of HCB and DDT, as well as the formation of bound residues, during sequestration were negligible. For the non-sterilized soil, over the initial 30 days of residence, the recoveries of HCB and DDT were 90–98% and 89–94%, respectively. However, when the residence time exceeded 30 days, the recoveries of HCB and DDT were decreased to 76–92% and 75–88%, respectively. The possible reasons are discussed in the following section.

3.2. Effect of biological activity on the distribution of HCB and DDT among SOM fractions

The distribution of HCB and DDT among various SOM fractions of the sterilized and non-sterilized soils with residence period is shown in Fig. 2. For the sterilized soil, as the residence time increased, HCB and DDT decreased in the FA, HA and BHA and increased in the lipid and IR fractions, suggesting a mass transfer of HCB and DDT from the

Fig. 2. Distribution patterns of HCB (a) and DDT (b) in each SOM fraction under ($\square$) sterilized and ($\circ$) non-sterilized regimes as a function of residence time.

FA, HA and BHA to the lipid and IR fractions. In the initial 30 days, there were no significant differences in the HCB and DDT distribution between the sterilized and non-sterilized soils. Nevertheless, significant decreases of HCB and DDT in the lipid and IR fractions of the non-sterilized regime were observed with increasing residence time from 90 to 300 days. This is in accordance with the low recoveries of total DDT and HCB concentrations.

Two possible reasons may be responsible for the low recoveries of DDT and HCB: biodegradation and the formation of “bound residues”. It is well documented that increased contact time led to increased sequestration and reduced biodegradation of HOCs (Bogan and Sullivan, 2003; Macleod and Semple, 2003). HOCs associated with humin fraction exhibited more resistance to desorption and have less bioavailability (Pignatello and Xing, 1996; Nam and Kim, 2002). Thus, if the biodegradation is the main reason, larger decreases of HCB and DDT should be found in the FA and HA fractions. However, this phenomenon was not observed in this study. In view of the low recoveries and the decrease of HCB and DDT in the lipid and IR fractions with an increasing contact time, we could deduce that the formation of bound residues rather

than biodegradation was the main explanation. Other studies also revealed that microbial activity may promote the formation of bound residue, thus decreasing the extractability and bioavailability of HOCs in soil (Carmichael et al., 1997; Guthrie and Pfaender, 1998; Macleod and Semple, 2003).

The mass transfer of DDT and HCB from FA, HA and BHA fit the two-compartment first-order kinetic model very well with all the R^2 higher than 0.97 (Table 1). A significant difference was found between rapid mass transfer rates (k_r) values, whereas slow mass transfer rate (k_s) values of non-sterilized soil were higher than that of sterilized soil. Both FA and HA fractions are believed to consist of two distinct regions for HOC sorption: the expanded (rubbery) region which is responsible for rapid mass transfer and the condensed (glassy) region which is responsible for slow mass transfer (Pignatello and Xing, 1996; Schaumann and Leboeuf, 2005). The unchanged k_r and increased k_s values suggest that the microbial activity could enhance HCB and DDT mass transfer by encouraging HCB and DDT to move into the condensed organic matrix. It has been reported that compared with those in sterile soil, HOCs in non-sterile soil showed greater levels of sequestration (more extensive decreases in extractability and bioavailability) (Carmichael et al., 1997; Richnow et al., 1998). Macleod and Semple (2003) found that there was a significant increase in the rate and extent of sequestration of pyrene in the biologically active soils than that in sterile soil. Guthrie and Pfaender (1998) also found that the extractability of pyrene in biologically active soils decreased to a greater extent than in NaN_3 -amended soil. Although there is growing observations of the role of microorganisms on the sequestration or mass transfer of HOCs in soil, the mechanisms are not fully understood. The possible mechanisms may be ascribed to the partial transformation (Richnow et al., 1997) and/or the carbon turnover activities by the soil microbial communities (Guthrie and Pfaender, 1998; Richnow et al., 1998; Howsam et al., 2001).

Table 1

Mass transfer rate parameters of HCB and DDT in the sterilized and non-sterilized soils

		Φ_r	k_r (days $^{-1}$)	k_s (days $^{-1}$)	R^2
<i>Sterilized</i>					
HCB	FA	$0.724 \pm 0.031a^*$	$0.644 \pm 0.051a$	$0.050 \pm 0.004a$	0.971
	HA	$0.706 \pm 0.023a$	$0.284 \pm 0.031a$	$0.004 \pm 0.001a$	0.987
	BHA	$0.694 \pm 0.047a$	$0.097 \pm 0.016a$	$0.002 \pm 0.001a$	0.978
DDT	FA	$0.811 \pm 0.033a$	$1.226 \pm 0.186a$	$0.058 \pm 0.010a$	0.986
	HA	$0.781 \pm 0.020a$	$0.273 \pm 0.012a$	$0.015 \pm 0.002a$	0.997
	BHA	$0.672 \pm 0.027a$	$0.237 \pm 0.021a$	$0.007 \pm 0.002a$	0.997
<i>Non-sterilized</i>					
HCB	FA	$0.707 \pm 0.025a$	$0.705 \pm 0.114a$	$0.071 \pm 0.004b$	0.976
	HA	$0.697 \pm 0.058a$	$0.257 \pm 0.045a$	$0.011 \pm 0.003b$	0.988
	BHA	$0.703 \pm 0.102a$	$0.108 \pm 0.067a$	$0.005 \pm 0.001b$	0.977
DDT	FA	$0.783 \pm 0.041a$	$1.512 \pm 0.123a$	$0.082 \pm 0.009b$	0.984
	HA	$0.802 \pm 0.053a$	$0.294 \pm 0.016a$	$0.021 \pm 0.003b$	0.991
	BHA	$0.658 \pm 0.047a$	$0.256 \pm 0.031a$	$0.013 \pm 0.002b$	0.977

*Values in the columns for the same compound followed by the same letter are not statistically different ($p > 0.05$).

3.3. Effect of soil humidity on the distribution of HCB and DDT among SOM fractions

Fig. 3 delineates the distribution of HCB and DDT among SOM fractions as a function of residence time under different soil moisture conditions. Their mass transfer rates calculated from Eq. (1) are listed in Table 2. As the soil water content increased from 1.9% to 30%, the mass transfer rates (k_r and k_s values) of HCB and DDT from FA, HA, and BHA fractions decreased obviously. When the water content increased from 30% to 60%, k_r and k_s values decreased further (with some exceptions). However, when the water content increased to 90%, no decreases but some increases of k_r and k_s values were found. The k_r and k_s values of FA, HA and BHA were the same or even higher than those of 1.9% water content. In the meantime, when the water content was 90%, the contents of HCB and DDT in the lipid fraction were the same as those of 60% water content, whereas HCB and DDT in the IR fraction were higher than those of 1.9% water content.

Previous studies suggested that humin provided the “internal surface” of a soil particle and rendered partially inaccessible to the bulk solution phase by overlayers of HA and FA

Fig. 3. Distribution patterns of HCB (a) and DDT (b) in each SOM fraction at (□) 1.9%, (○) 30%, (△) 60% and (▽) 90% of soil water content levels as a function of residence time.

Table 2

Mass transfer rate parameters of HCB and DDT for soils at different moisture levels

Water content		Φ_r	k_r (days ⁻¹)	k_s (days ⁻¹)	R^2
1.9%	HCB				
	FA	0.724 ± 0.031a*	0.644 ± 0.051a	0.050 ± 0.004a	0.971
	HA	0.706 ± 0.023a	0.284 ± 0.031a	0.004 ± 0.001a	0.987
	BHA	0.694 ± 0.047a	0.097 ± 0.016a	0.002 ± 0.001a	0.978
	DDT				
	FA	0.811 ± 0.033a	1.226 ± 0.086a	0.058 ± 0.010a	0.986
	HA	0.781 ± 0.020a	0.273 ± 0.012a	0.015 ± 0.002a	0.997
30%	BHA	0.672 ± 0.027a	0.237 ± 0.021a	0.007 ± 0.002a	0.997
	HCB				
	FA	0.603 ± 0.059b	0.231 ± 0.054b	0.011 ± 0.002b	0.981
	HA	0.649 ± 0.061a	0.100 ± 0.022b	0.002 ± 0.001a	0.964
	BHA	0.620 ± 0.026a	0.043 ± 0.003b	0.001 ± 0.000a	0.996
	DDT				
	FA	0.533 ± 0.059b	0.175 ± 0.045b	0.007 ± 0.001b	0.982
60%	HA	0.653 ± 0.055a	0.089 ± 0.017b	0.004 ± 0.001b	0.976
	BHA	0.636 ± 0.053a	0.100 ± 0.018b	0.003 ± 0.001b	0.983
	HCB				
	FA	0.662 ± 0.075b	0.099 ± 0.021c	0.006 ± 0.002c	0.956
	HA	0.657 ± 0.038a	0.048 ± 0.006c	0.001 ± 0.001a	0.973
	BHA	0.467 ± 0.052a	0.047 ± 0.011b	0.001 ± 0.000a	0.989
	DDT				
90%	FA	0.594 ± 0.054b	0.072 ± 0.013c	0.002 ± 0.001c	0.984
	HA	0.651 ± 0.032a	0.051 ± 0.005c	0.001 ± 0.001b	0.991
	BHA	0.456 ± 0.026b	0.056 ± 0.007c	0.001 ± 0.000c	0.990
	HCB				
	FA	0.914 ± 0.146a	0.785 ± 0.041d	0.089 ± 0.014a	0.971
	HA	0.867 ± 0.059d	0.367 ± 0.014d	0.017 ± 0.001d	0.987
	BHA	0.669 ± 0.078a	0.121 ± 0.056a	0.011 ± 0.001b	0.978
90%	DDT				
	FA	0.854 ± 0.077a	1.345 ± 0.037a	0.058 ± 0.015a	0.983
	HA	0.812 ± 0.109a	0.358 ± 0.032d	0.032 ± 0.003d	0.997
	BHA	0.739 ± 0.096a	0.346 ± 0.087a	0.012 ± 0.002a	0.992

*Values in the columns for the same compound followed by the same letter are not statistically different ($p > 0.05$).

(Weber and Huang, 1996; Bogan and Trbovic, 2003). Gradual contaminant migration into micropores presented in this portion, as well as the mineral portion itself, controlled the degree and rate of sequestration (Xing and Pignatello, 1997). When the soil moisture is raised, many of the micropores and mesopores of the fixed pore system are filled with water (Pignatello, 1998). Before organic compounds reach the sorption “sites” on these pores, they must travel through the water to reach the surfaces of pores. This would lead to an aqueous hindrance of organic compounds diffusing into soil pores or competition of organic compounds with water for specific sorption sites. As a result, when the soil moisture content increased, slower mass transfer of HCB and DDT from the FA, HA and BHA fractions were observed. On the contrary, when the soil moisture content was further increased to 90% of its water holding capacity, the conformation of FA and HA may be modified and became more swollen or expanded (Pignatello, 1998), resulting in a smaller degree of affinity, less non-linearity and hysteresis than the condensed humic materials (Xing and Pignatello, 1997; Feng et al., 2006). As BHA exhibited more similar characteristics to HA and may act as an outer layer

of soil organic matter, the same effect of water on HCB and DDT transfer from BHA may be observed. Under this circumstance, HCB and DDT were more readily transferred from the FA, HA and BHA fractions compared with the air-dried soil.

3.4. Effect of copper on the distribution of HCB and DDT among SOM fractions

Fig. 4 shows the distribution patterns of HCB and DDT among SOM fractions in the presence of different concentrations of coexisting Cu^{2+} . Their mass transfer rates calculated from Eq. (1) are listed in Table 3. As the concentration of Cu^{2+} added increased, the mass transfer rates (k_r and k_s values) of HCB and DDT from FA, HA, and BHA fractions decreased. Correspondingly, the contents of HCB and DDT in the lipid and IR fractions increased.

This may be attributed to the interactions between Cu^{2+} and SOM. It is suggested that the metallic cations could change the structure of FA and HA through complexation with functional polar groups of organic matter (such as $-\text{COOH}$ and $-\text{OH}$), leading to smaller, denser, and more

rigid molecules or molecular configurations (Maurice and Namjesnik-Dejanovic, 1999; Yuan and Xing, 2001; Feng et al., 2006), and rendering the reactive hydrophobic areas more accessible for HCB and DDT adsorption because of the coagulation of the most polar areas (Xing and Pignatello, 1997; Wrobel et al., 2003; Saison et al., 2004). The configuration-changed FA and HA possess glassy properties, higher affinity, greater sorption capacity and non-linearity, and high desorption hysteresis (Yuan and Xing, 2001; Wrobel et al., 2003; Feng et al., 2006). The penetration of HCB and DDT into these configuration-changed FA and HA contributed to the enhanced affinity of the FA and HA fractions for HCB and DDT and decreased the HCB and DDT mass transfer rate from these fractions with the increase of added Cu^{2+} . Although there are no reports dealing with the interaction between BHA and Cu^{2+} so far, based on the similar characteristics to HA, one can hypothesize that the added Cu^{2+} might have the same effect on BHA.

4. Conclusions

Although many studies have focused on the distribution of organic compounds among the FA, HA and humin fractions, direct observation of temporal distribution change of HCB and DDT in various SOM fractions especially in humin, under various environmental conditions such as microbial activity, soil moisture and coexisting metals is lacking. Based on the above study one can draw the following conclusions:

- (1) HCB and DDT mass transfer was primarily a physical process over the initial residence period of <30 days, with the activity of microbial communities becoming more obvious after 1 month of incubation, which accelerated the mass transfer;
- (2) Medium level of soil moisture (30% and 60% of water holding capacity) impeded HCB and DDT to transfer from the FA, HA and BHA to the lipid and IR fractions, whereas at high moisture level (90% of water holding capacity), the transfer rate was enhanced, implying that agriculture activity such as irrigation and natural events such as flooding in summer may influence the fate of HCB and DDT in the environment;
- (3) Coexisting Cu^{2+} slowed down the mass transfer of HCB and DDT from SOM fractions, suggesting that at locations where both heavy metal and organic compounds pollutions occurred, the mass transfer rate of HCB and DDT would be reduced with increasing contents of heavy metals. Thus the impacts of heavy metals should be taken into consideration when the transportation and fate of HOCs in the environment are to be predicted.

Although the fractionation procedure may somewhat alter the existing distribution of HCB and DDT in SOM fractions, this study was expected to provide additional convincing data on the contribution of different environmentally relevant conditions to the mass transfer of HCB and DDT in SOM, and

Fig. 4. Distribution patterns of HCB (a) and DDT (b) in each SOM fraction of soils with various concentrations of Cu^{2+} as a function of incubation time: ($\square$) 35.5 mg kg^{-1} , ($\circ$) 200 mg kg^{-1} , ($\triangle$) 500 mg kg^{-1} , and (∇) 1000 mg kg^{-1} .

Table 3

Mass transfer rate parameters of HCB and DDT for soils with various concentrations of Cu fit by two-compartment first order kinetic model

Cu ²⁺ added (mg kg ⁻¹)		Φ_r	k_r (days ⁻¹)	k_s (days ⁻¹)	R^2
0	HCB				
	FA	0.724 ± 0.031a*	0.644 ± 0.051a	0.050 ± 0.004a	0.971
	HA	0.706 ± 0.023a	0.284 ± 0.031a	0.004 ± 0.001a	0.987
	BHA	0.694 ± 0.047a	0.097 ± 0.016a	0.002 ± 0.001a	0.978
	DDT				
	FA	0.811 ± 0.033a	1.226 ± 0.086a	0.058 ± 0.010a	0.986
	HA	0.781 ± 0.020a	0.273 ± 0.012a	0.015 ± 0.002a	0.997
	BHA	0.672 ± 0.027a	0.237 ± 0.021a	0.007 ± 0.002a	0.997
200	HCB				
	FA	0.699 ± 0.055a	0.585 ± 0.062a	0.032 ± 0.008b	0.967
	HA	0.536 ± 0.053b	0.272 ± 0.081a	0.005 ± 0.001a	0.942
	BHA	0.668 ± 0.061a	0.089 ± 0.023a	0.001 ± 0.001a	0.931
	DDT				
	FA	0.809 ± 0.093a	0.913 ± 0.015b	0.004 ± 0.004b	0.958
	HA	0.840 ± 0.032a	0.169 ± 0.017b	0.008 ± 0.002b	0.992
	BHA	0.707 ± 0.072a	0.157 ± 0.038b	0.004 ± 0.001a	0.976
500	HCB				
	FA	0.608 ± 0.022b	0.413 ± 0.042b	0.018 ± 0.006b	0.965
	HA	0.684 ± 0.084b	0.067 ± 0.016b	0.001 ± 0.001b	0.957
	BHA	0.590 ± 0.041a	0.056 ± 0.008b	0.001 ± 0.001a	0.984
	DDT				
	FA	0.557 ± 0.064b	0.608 ± 0.107c	0.019 ± 0.006b	0.951
	HA	0.694 ± 0.064b	0.128 ± 0.026b	0.005 ± 0.002b	0.982
	BHA	0.757 ± 0.057a	0.082 ± 0.013c	0.002 ± 0.001a	0.982
1000	HCB				
	FA	0.795 ± 0.144b	0.101 ± 0.029c	0.009 ± 0.001b	0.912
	HA	0.719 ± 0.039b	0.045 ± 0.004b	0.001 ± 0.000b	0.993
	BHA	0.553 ± 0.033a	0.052 ± 0.006b	0.001 ± 0.000a	0.992
	DDT				
	FA	0.713 ± 0.085b	0.548 ± 0.222c	0.018 ± 0.008b	0.967
	HA	0.674 ± 0.077b	0.112 ± 0.029b	0.005 ± 0.002b	0.972
	BHA	0.722 ± 0.058a	0.084 ± 0.014c	0.002 ± 0.001a	0.978

*Values in the columns for the same compound followed by the same letter are not statistically different ($p > 0.05$).

to present a new angle to assess the multi-domain sorption concept.

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References

- Bogan, B.W., Sullivan, W.R., 2003. Physicochemical soil parameters affecting sequestration and mycobacterial biodegradation of polycyclic aromatic hydrocarbons in soil. *Chemosphere* 52, 1717–1726.
- Bogan, B.W., Trbovic, V., 2003. Effect of sequestration on PAH degradability with Fenton's reagent: roles of total organic carbon, humin, and soil porosity. *Journal of Hazardous Materials* 100, 285–300.
- Carmichael, L.M., Christman, R.F., Pfaender, F.K., 1997. Desorption and mineralization kinetics of phenanthrene and chrysene in contaminated soils. *Environmental Science and Technology* 31, 126–132.
- Chiou, C.T., Kile, D.E., Rutherford, D.W., Sheng, G., Boyd, S.A., 2000. Sorption of selected organic compounds from water to a peat soil and its humic acid and humin fractions: potential sources of the sorption nonlinearity. *Environmental Science and Technology* 34, 1254–1258.
- Cornelissen, G., Gustafsson, O., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environmental Science and Technology* 39, 6881–6895.
- Farrell, J., Grassian, D., Jones, M., 1999. Investigation of mechanisms contributing to slow desorption of hydrophobic organic compounds from mineral solids. *Environmental Science and Technology* 33, 1237–1243.
- Feng, X., Simpson, A.J., Simpson, M., 2006. Investigating the role of mineral-bound humic acid in phenanthrene sorption. *Environmental Science and Technology* 40, 3260–3266.
- Gaillardon, P., Dur, J.C., 1995. Influence of soil moisture on short-term adsorption of diuron and isoproturon by soil. *Pesticide Science* 45, 297–303.
- Gupta, S., Gajbhiye, V.T., 2002. Effect of concentration, moisture and soil type on the dissipation of flufenacet from soil. *Chemosphere* 47, 901–906.
- Gustafsson, O., Haghseta, F., Chan, C., Macfarlane, J., Gschwend, P.M., 1997. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environmental Science and Technology* 31, 203–209.
- Guthrie, E.A., Pfaender, F.K., 1998. Reduced pyrene bioavailability in microbially active soils. *Environmental Science and Technology* 32, 501–508.
- Hendershot, W.H., Duquette, M.A., 1986. Simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Science Society of America Journal* 50, 606–608.
- Howsam, M., Jones, K.C., Ineson, P., 2001. Dynamics of PAH deposition, cycling and storage in a mixed-deciduous (*Quercus-Fraxinus*) woodland ecosystem. *Environmental Pollution* 113, 163–176.

- Kang, S., Xing, B., 2005. Phenanthrene sorption to sequentially extracted soil humic acids and humins. *Environmental Science and Technology* 39, 134–140.
- Kohl, S.D., Rice, J.A., 1998. The binding of contaminants to humin: a mass balance. *Chemosphere* 36, 251–261.
- Macleod, C.J.A., Semple, K.T., 2003. Sequential extraction of low concentrations of pyrene and formation of non-extractable residues in sterile and non-sterile soils. *Soil Biology and Biochemistry* 35, 1443–1450.
- Maurice, P.A., Namjesnik-Dejanovic, K., 1999. Aggregate structures of sorbed humic substances observed in aqueous solution. *Environmental Science and Technology* 33, 1538–1541.
- Nam, K., Alexander, M., 1998. Role of nanoporosity and hydrophobicity in sequestration and bioavailability: tests with model soils. *Environmental Science and Technology* 32, 71–74.
- Nam, K., Kim, J.Y., 2002. Role of loosely bound humic substances and humin in the bioavailability of phenanthrene aged in soil. *Environmental Pollution* 118, 427–433.
- Nam, K., Kim, J.Y., Oh, D.I., 2003. Effect of soil aggregation on the biodegradation of phenanthrene aged in soil. *Environmental Pollution* 121, 147–151.
- Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis, Part 2*, second ed. ASA and SSSA, Madison, WI, pp. 539–579.
- Niklinńska, M., Chodak, M., Laskowski, R., 2006. Pollution-induced community tolerance of microorganisms from forest soil organic layers polluted with Zn or Cu. *Applied Soil Ecology* 32, 265–272.
- Pignatello, J.J., 1998. Soil organic matter as a nanoporous sorbent of organic pollutants. *Advances in Colloid and Interface Science* 76–77, 445–467.
- Pignatello, J.J., Xing, B., 1996. Mechanisms of slow sorption of organic chemicals to natural particles. *Environmental Science and Technology* 30, 1–11.
- Qiu, X.H., Zhu, T., Li, J., Pan, H.S., Li, Q.L., Miao, G.F., Gong, J.C., 2004. Organochlorine pesticides in the air around the Taihu Lake, China. *Environmental Science and Technology* 38, 1368–1374.
- Richnow, H.H., Eschenbach, A., Mahro, B., Seifert, R., Wehrung, P., Albrecht, P., Michaelis, W., 1998. The use of ^{13}C -labelled polycyclic aromatic hydrocarbons for the analysis of their transformation in soil. *Chemosphere* 36, 2211–2224.
- Richnow, H.H., Seifert, R., Hefter, J., Link, M., Francke, W., Schaefer, G., Michaelis, W., 1997. Organic pollutants associated with macromolecular soil organic matter: mode of binding. *Organic Geochemistry* 26, 745–758.
- Saison, C., Perrin-Ganier, C., Amellal, S., Morel, J.-L., Schiavon, M., 2004. Effect of metals on the adsorption and extractability of ^{14}C -phenanthrene in soils. *Chemosphere* 55, 477–485.
- Schaumann, G., Leboeuf, E.J., 2005. Glass transitions in peat: their relevance and the impact of water. *Environmental Science and Technology* 39, 800–806.
- Wang, Y.P., Shi, J.Y., Wang, H., Lin, Q., Chen, X.C., Chen, Y.X., 2007. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. *Ecotoxicology and Environmental Safety* 67, 75–81.
- Watanabe, N., Schwartz, E., Scow, K.M., Young, T.M., 2005. Relating desorption and biodegradation of phenanthrene to SOM structure characterized by quantitative pyrolysis GC-MS. *Environmental Science and Technology* 39, 6170–6181.
- Weber, W.J., Huang, W., 1996. A distributed reactivity model for sorption by soils and sediments. 4. Intraparticle heterogeneity and phase-distribution relationships under nonequilibrium conditions. *Environmental Science and Technology* 30, 881–888.
- Wrobel, K., Sadi, B.B.M., Wrobel, K., Castillo, J.R., Caruso, J.A., 2003. Effect of metal ions on the molecular weight distribution of humic substances derived from municipal compost: ultrafiltration and size exclusion chromatography with spectrophotometric and inductively coupled plasma-MS detection. *Analytical Chemistry* 75, 761–767.
- Xing, B., Pignatello, J.J., 1997. Dual-mode sorption of low-polarity compounds in glassy poly (vinyl chloride) and soil organic matter. *Environmental Science and Technology* 31, 792–799.
- Yuan, G., Xing, B., 2001. Effects of metal cations on sorption and desorption of organic compounds in humic acids. *Soil Science* 166, 107–115.
- Zhang, J.-j., Wen, B., Shan, X.-q., Zhang, S., Khan, S.U., 2007. Temporal change in the distribution patterns of hexachlorobenzene and dichlorodiphenyltrichloroethane among various soil organic matter fractions. *Environmental Pollution* 150, 234–242.